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ANGULAR-LEAFSPOT OF TOBACCO, AN UNDESCRIBED BACTERIAL DISEASE[1]

By F. D. FROMME, *Plant Pathologist and Bacteriologist*, and T. J. MURRAY,[2] *formerly Associate Bacteriologist, Virginia Agricultural Experiment Station*

INTRODUCTION

About the first of August, 1917, the Virginia Experiment Station received a petition from 52 tobacco growers in Halifax County, asking assistance in combating a tobacco disease which threatened serious losses to the crop. Diseased tobacco plants (*Nicotiana tabacum*) were later received from a correspondent at South Boston, the leaves of which were covered with spots which were different from any previously seen by us, the most distinctive feature being the irregularly angular shape. Numerous motile bacteria were found in crushed tissue mounts and freehand sections of spots, and the organism was readily obtained in pure culture from poured plates of beef-peptone agar. The same organism was obtained later from material which the writers collected in the field from five different places in Halifax County.

Several inspection trips were made during the remainder of the season of 1917. On August 10 the disease was found on the tobacco plants in most of the fields along the road between South Boston and Republican Grove, a distance of 30 miles. It was found later in the northern part of Halifax County, at Clarkton and other points, in the southern part of Campbell County, at Brookneal and Naruna, and at Charlotte Court House, in Charlotte County. Inquiries through county agents extended the distribution to include Mecklenburg, Pittsylvania, Henry, and Patrick Counties, involving the greater part of the flue-cured-tobacco belt in Virginia.

FIELD APPEARANCE OF THE DISEASE

The epiphytotic was well advanced by August 10. Many fields were found in which practically every plant was affected, and in some fully 50 per cent of the crop was estimated by the growers to be unfit for harvest. Late plantings were found to be less severely spotted than the early plantings, and the most forward and vigorous plants were invariably more seriously affected than the less vigorous ones.

The distribution of the spots on the plants was a distinctive feature and one that readily separated them from "frogeye" (caused by *Cerco-*

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spora nicotianae E. and E.). The heaviest spotting was found on the top and middle leaves, while the bottom or sand leaves were but slightly affected. Frogeye, on the contrary, is found chiefly on the sand leaves. The field evidence indicated that the vertical distribution of the spots on the plants was determined by their stage of growth at the time of infection, and that leaves which had attained a certain stage of growth were not susceptible to infection. In some fields the infection was heaviest on the top leaves and in others on the middle leaves. Frequently the spots were found to be most numerous on one side of the plants, indicating the probable dissemination of the inoculum by wind-blown rain.

The consensus of the statements of the farmers placed the first appearance of the spotting between the third and fourth weeks in July, following a protracted period of rainfall. The tobacco at this time was at about the stage for topping. No one had seen any of the spotting earlier in the season, and there had been no evidence of it in the seed beds. One farmer who had planted two fields from the same seed bed stated that the disease was much worse in the field on new ground than on old ground. There was no evidence from any source that continuous cropping with tobacco, as practiced on many farms, had been conducive to heavier infection.

Some of the farmers had been troubled with the disease in the previous year, 1916, and a few stated that they had seen it occasionally over a period of 10 or 12 years, but never so generally destructive as in 1917. Prof. T. B. Hutcheson, of the Virginia Experiment Station, informs us that he has known this spotting in Charlotte County for 12 or 15 years.

Opinions among the farmers as to the cause of the disease were centered on the wet weather, the fertilizer, especially the supposed deficiency in potash, and the seed. The disease was found on both the flue-cured and sun-cured types of tobacco, and no differences in the susceptibility of varieties were apparent.

Evidence that the disease is not occasioned by lack of potash and that it is not a fertilizer phenomenon, except in a secondary way, was obtained from a study of the distribution of the spotting on the fertilizer plots at the experimental substation at Charlotte Court House. Early in September the disease was prevalent on all plots which had received applications of acid phosphate alone or in various combinations with sulphate of potash and nitrate of soda. It was the opinion of several observers who went over these plots that they were uniformly spotted. None of the spotting was present, however, at this time, on any plots which had had no applications of phosphorus and which had received only nitrate of soda or sulphate of potash, or both. There was no spotting on the control plots. The tobacco plants on those plots which had received phosphorus were larger, more vigorous, and matured three weeks earlier than those which had had no phosphorus. The superintendent of

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the substation informed us that the tobacco on these backward plots became spotted later with the advent of additional rainfall and cool nights, and that the second growth tobacco from the early harvested plots also became diseased.

CHARACTER OF LOSSES

A demonstration acre of tobacco at the substation at Charlotte Court House was severely spotted in 1917, much more so than in any previous years. The yields from this acre were obtained to determine the variation in yield and grade between 1917 and the average of preceding years. The data obtained are included in Table I. The yield of the average year was determined from demonstration acres for the years 1913, 1914, and 1915. Complete data for 1916 were not available, but the total yield for this year, 1,005 pounds, shows a close agreement with the three preceding years. The effect of the disease is shown both in a reduction in yield and in grade. The yield from the acre in 1917 was 212 pounds less than that of the average year, or approximately 80 per cent of the average. The yields in the different grades for 1917 show a loss in weight in all of the three highest grades and a strong increase in the lowest grade. There were approximately 40 per cent more sand lugs in 1917, in proportion to the higher grades, than in the average year, and the percentage of longs was slightly greater. The gain in the sand lugs was made up by losses in the two middle grades, good lugs and shorts. The total loss in weight was 20 per cent, and the loss in grade approximately 40 per cent.

TABLE I.—*Variation in yield and grade between severely spotted tobacco in 1917 and the average of three preceding years of light spotting*

Grade.	1917	Average year.	1917	Average year.	Difference between 1917 and average year.	
	Pounds.	*Pounds.*	*Per cent.*	*Per cent.*	*Pounds.*	*Per cent.*
Sand lugs	495	205	58. 9	19. 4	+290	+39. 5
Good lugs	.....	290	.....	27. 6	−290	−27. 6
Shorts	155	346	18. 5	32. 9	−191	−14. 4
Longs	190	211	22. 6	20. 1	− 21	+ 2. 5
Total	840	1, 052	.....	.....	−212	−20. 2

DESCRIPTION OF THE SPOTS

The spots are found only on the leaves, none on stems or floral parts. They are scattered over the entire leaf surface, between the larger veins, or are crowded in irregular groups. They are usually bordered by viens which act as barriers against further enlargement. More than 500 spots may be counted on the average leaf of a heavily infected plant. They are about equally prominent on the upper and lower leaf surfaces. The

size of the spots varies from a pinhead up to 8 mm. in diameter at the widest part, and the average diameter of the mature spot is 4 mm. The most striking feature is the irregularly angular shape and the uneven jagged outline (Pl. 25-27). The center of the spot is tan or reddish brown, with a darker thin border and often a suggestion of zonation which is never conspicuous. A small, dark center is usually seen. In earlier stages the spots are darker in color, almost black [1] at first, and are circular or only slightly angular (Pl. 25, A). The center of the spot becomes dry and thin with age, paler in color until almost white, and may drop out, leaving irregular holes which are sometimes so numerous with the confluence of spots that a skeleton leaf composed only of the viens remains. Most of the tobacco is harvested before this stage is reached. No sharply defined halo is present, but a narrow clear zone on the border is seen by transmitted light. The tissue bordering the spots is yellowed, but this diffuses gradually into the normal green. Usually the spots are more numerous on one side of the midrib.

In stained sections of fixed material the organism is found in great numbers throughout the shrunken tissue included in the spot. It is found both within and between the cells, and vacuolated cells are completely filled with it.

INOCULATION EXPERIMENTS

Proof of the pathogenecity of the organism isolated from leafspot material was obtained through inoculations on seedling tobacco plants (Warne variety). This work was carried on in the greenhouse during the winter months. In all, some 150 plants were inoculated, and fully 96 per cent developed spots. All five isolations of the organism produced infection. Infection was readily obtained by atomizing the plants with aqueous suspensions of the organism, by swabbing the leaves with the cotton plug of bouillon cultures and by puncture with contaminated needles. It was found necessary to place the plants in moist chambers for 24 hours subsequent to inoculation. Some plants which were inoculated and left in the open greenhouse failed to develop any spots, and none of the many control plants became infected. No leaf injury is necessary for infection. Apparently the organism gains entrance through the stomata on either the upper or lower surface of the leaf. The inoculum has been recovered in pure culture a number of times, and these recoveries have been used for reinfections. Secondary infections have never developed, but this is a common experience with plant pathogens under the dry atmospheric conditions of the greenhouse.

As many as 200 spots were obtained on a single leaf through inoculation with the atomizer, but the average ran much lower than this. Still heavier infections were obtained by swabbing with contaminated cotton plugs. The spots obtained were typical of the spots seen in the field, but were smaller. They averaged about 1.5 mm. in diameter, and

[1] One farmer stated that his first impression of the spot was that someone had spattered ink on the leaves.

many were no larger than 0.5 mm., mere pin pricks. The spots were frequently grouped on a limited area of the leaf and were often found on one side of the midrib only.

The discrepancy in size between the field spots and those developed in the greenhouse is apparently due to the difference in the size and vigor of the plants. The plants did not grow vigorously in the greenhouse and at maturity were not more than half as large as plants in the field. The largest spots in the greenhouse were always found on the most vigorous plants and on the most rapidly growing leaves. The incubation period was also shortest with the same plants and leaves. This varies between 4 and 10 days, with the average about 7 days. When plants which have developed a number of leaves are inoculated, the first spots are seen on one or two leaves near the top and intermediate in age. Later, within a few days, spots may appear on two or three older leaves immediately below these, but no spots develop on old, full-grown leaves, nor on very young ones. Young leaves become infected, however, when the inoculum is rubbed in with the fingers or a cotton plug. The young leaves are closely set with trichomes, and these seemingly serve as a mechanical protection against inoculation with the atomizer; the spray is caught and retained on them. The spots attain their maximum size within a few days after their appearance. They are largest on the younger leaves and may be mere pin pricks or flecks on the older leaves.

TABLE II.—*Results from the inoculation of tobacco plants with the angular-leafspot organism*

Plant No.	Number of spots per leaf on leaf No.[a]				
	1	2	3	4	5
1	.....	2	.....	.....	.....
2	.....	14	13	1	.....
3	1	1	.....	2	.....
4	5	68	19	**33**	.....
5	.....	.....	5	1	.....
6	14	29	17	21	**9**
7	157	8	39	.....	**30**
8	.....	.....	51	14	**6**
9	.....	44	63	23	.....
10	1	1	5	.....	.....
11	1	18	.....	.....	.....
12	.....	.....	.....	.....	.....
13	3	5	4	**41**	.....
14	6	3	4	.....	.....
15	.....	.....	.....	.....	.....
16	1	1	.....	.....	.....
17	58	.....	37	.....	.....
18	30	.....	23	14	.....
Total spots	277	193	280	76	.....
Total flecks	.....	.....	.....	**74**	**45**

[a] Leaves are numbered from the top downward. The plants bore from 10 to 14 leaves. Bold-face figures indicate flecks or spots that are very small and are visible only in transmitted light.

The records of one inoculation series are given (Table II) chiefly to show the vertical distribution of the spots with reference to the position of the leaves and their relative ages. These plants were inoculated on January 16, 1918, with an atomizer containing an aqueous suspension of a strain of the angular-leafspot organism isolated from material from Republican Grove, Va., on August 10. The plants were covered with moist chambers for 12 hours subsequent to inoculation. They were at the blooming stage and were topped just before inoculation. The spots were first visible on January 22, and the counts were made on February 1.

Table II shows that the spots were about equally distributed over the first, second, and third leaves, with a few on the fourth leaves and none on those older and lower on the stem. Flecks developed on the fourth and fifth leaves, but not on younger or older leaves.

COMPARISON OF ANGULAR-LEAFSPOT WITH OTHER LEAFSPOTS OF TOBACCO

The angular-leafspot can not be assigned with certainty to any of the previously described tobacco leafspots of bacterial causation. It has some features in common with the "whitespot" of Delacroix (*2*, *3*),[1] caused by *Bacillus maculicola* Del., but the descriptions of this disease and of the organism are too meager to afford an adequate basis for comparison. "Blackrust," a disease of Deli tobacco described by Honing (*4*), differs from angular-leafspot in several important features, and the causative organism, *Bacterium pseudozoogloeae* Honing, is readily distinguished from the angular-leafspot organism.

The spot which Wolf and Foster (7) have recently described under the name "wildfire" from North Carolina appears to differ strikingly from the disease under discussion. The most noteworthy points of difference being found in the broad, distinct halo which borders the wildfire spots, in their circular form and zonated interior, and in size. Wildfire spots are 2 to 3 cm. in diameter, while those of the angular-leafspot are only 4 mm., on the average. Some contrasting features between *Bacterium tabacum* Wolf and Foster, the wildfire organism, and the angular-leafspot organism are given in Table III. Features in common between the two diseases are found in their sudden appearance and rapidity of spread, and in the relation between rainfall and epiphytotics, although this seems a common feature of bacterial leafspots of tobacco. It seems quite probable that the disease to which Wolf and Foster refer as "speck" is identical with our angular-leafspot. They state that speck is caused by a lack of potash.

[1] Reference is made by number (italic) to "Literature cited," p. 227–228.

TABLE III.—*Comparison of Bacterium angulatum and Bact. tabacum*

Bact. angulatum.	*Bact. tabacum.*
1. Size, 0.5×2–2.5 μ.	Size, 1.2×3.3 μ.
2. 3 to 6 polar flagella.	1 polar flagellum.
3. Liquifies gelatin rapidly.	Liquifies gelatin slowly.
4. Forms acid with saccharose and dextrose.	Forms acid with saccharose, dextrose, lactose, and glycerin.
5. No growth in closed arm of fermentation tubes.	Growth in closed arm of fermentation tubes containing dextrose and saccharose.

An unsigned note in the Yearbook of the Virginia Department of Agriculture and Immigration (*5*) contains a report of a field investigation of a spotting of tobacco leaves (evidently angular-leafspot) which was prevalent in Pittsylvania and Mecklenburg counties in 1917. The spotting is assigned to microorganisms, and it is stated that the disease becomes serious only under certain conditions which affect the resistance of the tobacco plant. The most important of these are considered to be rainfall and excess of nitrogen in the fertilizer or soil. A liberal supply of potash is said to decrease the severity of the disease, but considerable damage was noted even with heavy potash applications. The same disease is said to occur in North Carolina and Maryland.

Two other leafspots, the causes of which have not been assigned to bacteria, and which appear somewhat similar to angular-leaf spot, are "rust" of tobacco in Connecticut (*1, p. 366–367, pl. 31, b, c*) and *Pockenkrankheit* (*6, p. 56*) of tobacco in Europe. Clinton's figures of rust, especially that shown in his Plate 31, c, look much like angular-leafspot. He states that rust is usually found on leaves affected with calico and believes it may be caused by scorching of the sun. *Pockenkrankheit* is ascribed to excessive transpiration accompanying decreased water supply.

OCCURRENCE OF THE DISEASE

It seems quite probable that angular-leafspot is a disease of rather general distribution and one of long standing which has not been sufficiently destructive to attract extensive notice, except in seasons unusually favorable for its development. Our experience indicates that rainfall accompanied by subnormal temperatures favors infection by the leafspot organism and that any combination of conditions which promotes a rapid, succulent growth of the host favors the development of the organism within the leaf tissue. It seems quite probable that infection may be common in some seasons, but that in the absence of the predisposing growth factors little development ensues, and no damage results.

DESCRIPTION OF THE ORGANISM

The caustive organism of angular-leafspot appears to be an undescribed species, and a description is therefore appended.

Bacterium angulatum, n. sp.

As it occurs in the plant and also on media, the organism is a short rod with rounded ends, single or in pairs, 0.5 μ wide by 2 to 2.5 μ long. No spores are produced, and no capsules have been demonstrated. It is motile by means of a small tuft of flagella at one pole, demonstrated by the Van Ermengen silver-nitrate method. The number of flagella varies from about three to six, and they are slightly longer than the body of the bacterium. It stains readily with the ordinary dyes, and is Gram-negative and not acid-fast.

TEMPERATURE RELATIONS

The best growth is obtained at temperatures between 17° and 20° C. There was no growth at 37.5° C.

CULTURAL CHARACTERS

AGAR PLATES.—Colonies are visible in 48 hours at 20 to 22° C. They increase slowly in size, being less than 1 mm. in diameter at three days. After seven days the largest are 4 mm. in diameter and the average about 3 mm. The maximum size attained is 8 mm. They are round, smooth, convex, shining, opalescent at first, later becoming dull white with a slight creamy cast and develop an opaque center with a clear margin. They are finely granular under the compound microscope, with a slightly undulate margin. Buried colonies are lenticular.

AGAR SLANTS.—Growth is slight in three days, the line of the stroke being about 1 mm. broad, and is not more than 5 mm. broad after one month. The growth is filiform, slightly raised, shining, smooth, and slimy. Considerable white sediment is formed at the base. The medium attains a slight pale-green fluorescence.

GELATIN PLATES.—Colonies are visible in 48 hours as small points similar to those on agar. Liquefaction is rapid, beginning in cuplike hollows in 48 hours. The cupules are 5 to 10 mm. broad in 3 days. Thickly sown plates are completely liquefied in 48 hours.

GELATIN STABS.—Liquefaction is infundibuliform and begins in 24 hours. As liquefaction progresses, the upper part becomes stratiform, and the lower maintains the blunt funnel form. Liquefaction is complete within 15 days at 18° to 20° C.

BEEF BOUILLION.—Uniform heavy clouding occurs within 48 hours. No surface scum or pellicle is produced, and there are no zoogleæ. A grayish precipitate forms in old cultures.

BEEF BOUILLION WITH SODIUM CHLORID.—Two per cent sodium chlorid produced only a slight inhibition of growth in 48 hours. Heavy clouding was present at seven days with 2 per cent of sodium chlorid, but growth was practically inhibited with 4 per cent of the salt.

POTATO CYLINDERS.—The form of growth is similar to the agar slant but with a slight dull yellow pigment.

MILK.—Inoculated milk clears slowly and without coagulation. The protein is digested. Clearing begins within seven days in definite layers from the top downward, and is complete within three weeks. The liquid is only faintly translucent at this time and is near Ridgeway's pale fluorite green.

LITMUS MILK.—Lavender-colored litmus milk becomes blue from the top downward in definite layers. The color change begins on the second or third day and is complete within 14 days. During two months the medium remained dark blue and liquid.

FERMENTATION TUBES.—The tests were made in basal solutions of 1 per cent peptone, to which was added 1 per cent of the following carbon compounds: Saccharose, dextrose, lactose, maltose, glycerin, and mannit. Clouding occurred in the open ends of all tubes in 48 hours, but the closed ends remained clear with a distinct line across the inner part of the U. Tests with neutral litmus paper gave an acid reaction with saccharose and dextrose, while the others were neutral or faintly alkaline. No gas was formed with any of the compounds.

The tests for acid production with dextrose and saccharose were repeated, with 100-cc. portions of 2 per cent of each in 2 per cent peptone water. After 10 days the reaction was determined with phenolphthalein as the indicator. Both solutions showed acid production in excess of the controls as follows: Saccharose control, +6.6; saccharose inoculated, +11.0; dextrose control, +8.8; dextrose inoculated, +12.6.

REDUCTION OF NITRATES.—Nitrates are not reduced.

INDOL.—A moderate indol production was obtained in Dunham's solution.

USCHINSKY'S SOLUTION.—Clouding was evident after 48 hours and was only moderate at seven days. The medium did not change color and no scum or pellicle was formed.

AEROBISM.—The organism appears to be strictly aerobic.

Following the chart of the Society of American Bacteriologists, the group number is 211.23220 [1] 33.

SUMMARY

A leafspot disease of tobacco which was prevalent in the flue-cured belt in Virginia in 1917 is described under the name "angular-leafspot." The disease has apparently been present to some extent for several years and may have a wide distribution.

The disease is caused by a specific organism, which is described as "*Bacterium angulatum*." Rainfall is an important aid to infection, and the development of the organism within the tobacco leaf is apparently dependent to a marked degree on those predisposing factors which promote a rapid, vigorous growth of the host.

The disease produced losses in both yield and grade. These were calculated in one field as a 20 per cent reduction in yield and a 40 per cent reduction in grade.

LITERATURE CITED

(1) CLINTON, G. P.
1915. CHLOROSIS OF PLANTS WITH SPECIAL REFERENCE TO CALICO OF TOBACCO. *In* Conn. Agr. Exp. Sta. Ann. Rpt. 1914, p. 357–424, pl. 25–32.

(2) DELACROIX, Georges.
1905. LA ROUILLE BLANCHE DU TABAC ET LA NIELLE OU MALADIE DE LA MOSAÏQUE. *In* Compt. Rend. Acad. Sci. [Paris], t. 140, no. 10, p. 678–680.

(3) ———
1906. RECHERCHES SUR QUELQUES MALADIES DU TABAC EN FRANCE. *In* Ann. Inst. Nat. Agron., s. 2, t. 5, p. 141–232, 17 fig.

(4) HONING, J. A.
1914. DE "ZWARTE ROESTE" DER DELI-TABAK. (BLACK RUST OF DELI-TOBACCO.) Bul. Deli-Proefstat. 1, 16 p., 2 pl. Literatuur, p. 16.

[1] A very slight fluorescence is imparted to the medium with agar stroke.

(5) LEAFSPOT DISEASE OF TOBACCO.
1918. *In* Va. Dept. Agr. and Immigr. Yearbook, 1917/18, (Bul. 126), p. 55–56.
(6) PETERS, Leo, and SCHWARTZ, Martin.
1912. KRANKHEITEN UND BESCHÄDIGUNGEN DES TABAKS. Mitt. K. Biol. Anst. Land. u. Forstw. 13, 128 p., 92 fig.
(7) WOLF, Frederick A., and FOSTER, A. C.
1918. TOBACCO WILDFIRE. *In* Jour. Agr. Research, v. 12, no. 7, p. 449–458, 2 fig., pl. 15–16. Literature cited, p. 458.

PLATE 25

A.—A tobacco leaf showing an early stage of the angular-leafspot.
B.—Angular leaf spots on a tobacco leaf. About natural size.

A

B

A

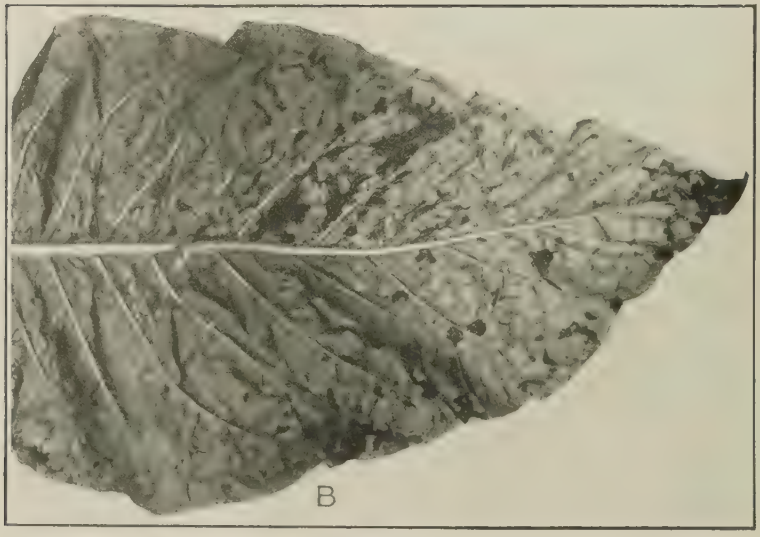

B

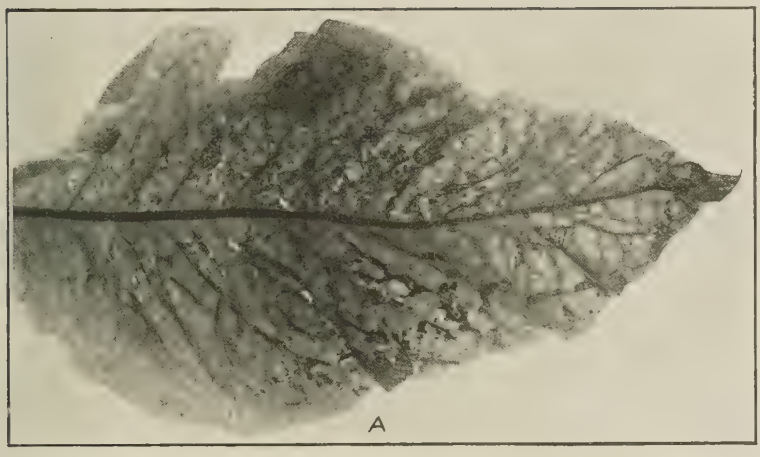
PLATE 26

A.—Upper surface of a tobacco leaf affected with the angular-leafspot.
B.—Lower surface of a tobacco leaf affected with the angular-leafspot.

PLATE 27

A.—Angular leaf spots on a tobacco leaf as seen in transmitted light.

B.—Atypical angular leaf spots on a narrow leaf of tobacco. The spots are blanched and rounded.



A

B